DE 19,750,422 C1

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products of the prior art. The agents can be administered without problems, even orally in high doses, by encapsulation in gelatins.

The invention concerns the use of phytostenol esters, optionally together with selected potentiation agents to produce agents to reduce cholesterol content in the serum of warm-blooded animals.

Hypocholesterolemic active ingredients are understood to mean agents that lead to a reduction of cholesterol content in the serum of warm-blooded animals without causing an inhibition or reduction of the formation of cholesterol in the blood in so doing. Phytostenols, i.e., plant stenols, and their fatty acids esters have already been proposed for this purpose by Peterson et al., in J. Nutrit. 50, 191 (1953). US Patents 3,089,939 and 3,203,862 and German Laid-open Patent DE-OS 20 35 069 (Procter & Gamble) also pointed in the same direction. The active ingredient is ordinarily added to cooking or edible oils and then absorbed via the nutrition, but during which the employed amounts are generally limited and usually lie below 0.5 wt% in order to prevent turbidity of the edible oil or precipitation of the stenols on the addition of water. Storage-stable emulsions of stenol esters in sugar or polyglycerol esters are proposed in European Patent Application EP-A1 0289636 (Ashai) for use in the food area, cosmetics, pharmaceutical preparations and in the agricultural sector. Incorporation of phytostenol esters to reduce blood cholesterol compounds in margarine, butter, mayonnaise, salad dressing, etc., is proposed in European Patent EP-B1 0594612 (Raision).

However, a shortcoming is that the phytostenol esters ordinarily can only be added to foods in limited amounts, since otherwise there is a hazard that they will adversely affect the taste and/or consistency of the food. However, for subsequent influencing of cholesterol content in the blood, the intake of larger amounts of phytostenol esters will be desirable. The rate at which the substances reduce the content of cholesterol in the serum was also in need of improvement. The task of the invention therefore consisted of eliminating these deficiencies.

The object of the invention is the use of esters of phytostenols with fatty acids with 6-24 carbon atoms and at least two conjugated double bonds, optionally together with potentiation agents chosen from the group formed of tocopherols, chitosans, phytostenol sulfates and/or (deoxy)ribonucleic acids, to produce hypocholesterolemic agents.

It was surprisingly found that phytostenol esters based on conjugated fatty acids had significantly higher activity during reduction of cholesterol content in the blood than comparable phytosterol esters derived from saturated fatty acids, singly unsaturated fatty acids or a multiply unsaturated fatty acids with two or more unconjugated double bonds. By combining the phytostenol esters to be used according to the invention (component a) with potentiation agents (component b) from the group of chitosans, phytostenol sulfates, and/or deoxy- or ribonucleic acids, which themselves have no or only very limited hypocholesterolemic properties, reduction

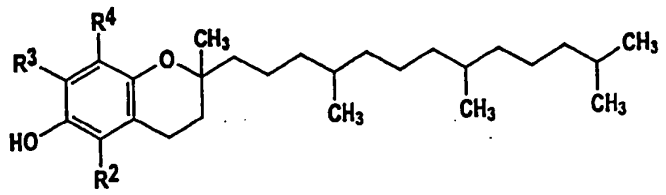
of the cholesterol content in the serum can be further accelerated. Both the phytostenol esters and the active ingredient mixtures can also be taken orally encapsulated in gelatin without problems.

Phytostenol esters

Phytostenols (or the synonym phytosterols) are understood to mean plant steroids that carry a hydroxyl group only on C-3 but otherwise have no functional groups. The phytostenols generally have 27-30 carbon atoms and a double bond at 5/6, optionally 718 [sic; 7/8], 8/9, or other positions. The corresponding saturated stanols can be obtained from the unsaturated stenols by hydrogenation, which are also a part of the invention. By esterification of stenols or stanols with unsaturated fatty acids with conjugated double bonds, preferably conjugated linoleic acid (CLA) or conjugated fish fatty acids, substances that form component a are obtained. The phytostenol component of the esters can be derived from ergostenols, campestenols, stigmastenols, brassicastenols, as well as preferably sitostenols or sitostanols and especially β-sitostenols or β-sitostanols. Production can occur in the known manner, e.g., by direct esterification of stenols with the fatty acids and subsequent hydrogenation of the ester, by direct esterification of stanols with fatty acids or preferably by transesterification and optionally hydrogenation of stenols or stanols with the corresponding conjugated fatty acid methyl esters. A general production method for transesterification of stenols/stanols with fatty acid lower alkyl esters or triglycerides in the presence of the appropriate catalysts, e.g. sodium methylate or especially enzymes, is described in EP A2 0195311 (Yoshikawa). The fatty acid component of the phytostenol esters according to the invention can also contain in lesser amounts (<50 mol%), saturated, monounsaturated, or polyunsaturated, unconjugated fractions. As a result, a technical mixture with a high fraction of conjugated linoleic acid can be used to produce the esters, instead of pure conjugated linoleic acid, this industrial mixture being available under the name Selin® CLA (Grünau). Corresponding fatty acid methyl esters or triglycerides (e.g., Selin® CLA-TG) with a high content of conjugated acid can also be transesterified in the same manner to produce phytostenol esters.

Tocopherols

Tocopherols, which are considered as the potentiation agent for phytostenol esters, are understood to mean chroman-6-oles (3,4-dihydro-2-H-1-benzopyran-6-oles) substituted in position 2 with 4,8,12-trimethyltridecyl groups, which have the following formula (II):

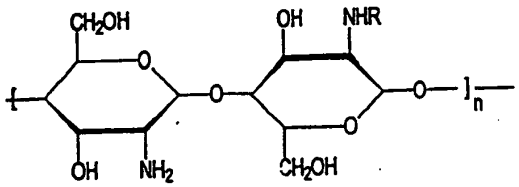


(II)

in which R^2, R^3, and R^4 independent of each other represent hydrogen or a methyl group. Tocopherols are among the bioquinones, i.e., polyprenylated 1,4-benzo- or naphthoquinones whose prenyl chains are more or less strongly saturated. Typical examples of tocopherols considered according to the invention as component (b1) are ubiquinones, boviquinones, K_5 Vitamin and/or menaquinones (2-methyl-1,4-naphthoquinones). α-, β-, γ-, δ- and ε-tocopherols are also distinguished among the tocopherols, the latter still having the original unsaturated prenyl side chain, as well as α-tocopherolquinone and -hydroquinone, in which the pyran ring system is open. α-Tocopherol (Vitamin E) of formula (II) in which R^2, R^3 and R^4 represent methyl groups, or esters of α-tocopherol with carboxylic acids with 2-22 carbon atoms, e.g., α-tocopherol acetate or α-tocopherol palmitate, are preferably used as component (b).

Chitosans

Chitosans, which are optionally considered as potentiation agent (b2) for the phytostenol esters, represent biopolymers and are included in the group of hydrocolloids. Chemically they are partially deacetylated chitins of different molecular weights containing the following (idealized) monomer component (III):



(III)

In contrast to most hydrocolloids that are negatively charged in the range of biological pH values, chitosans represent cationic biopolymers under these conditions. The positively charged chitosans can interact with oppositely charged surfaces and are therefore used in cosmetic hair and body care agents, as well as pharmaceutical preparations (see Ullmann's Encyclopedia of Industrial Chemistry, 5th edition, Vol. A6, Weinheim, Verlag Chemie, 1986, pp. 231-332). Reviews concerning this subject have also been published by B. Gesslein et al. in HAPPI 27, 57 (1990), O. Skaugrud in Drug Cosm. Ind. 148, 24 (1991) and E. Onsoyen et al. in Seifen-Ole-Fette-Wachse 117, 633 (1991). In the production of chitosans one starts from chitin,

preferably the shell residues of crustaceans, which are available in large amounts as a cheap raw material. Chitin is ordinarily deproteinated in a process first described by Hackmann et al., ordinarily by the addition of bases, demineralized by the addition of mineral acids and finally deacetylated by the addition of strong bases, in which the molecular weights can be distributed over a broad range. Either low-molecular chitosans with an average molecular weight of about 50,000 to about 250,000 dalton or high-molecular chitosans with an average molecular weight of about 500,000 to about 2,000,000 are preferably used. Corresponding methods are known, e.g., from Makromol. Chem. 177, 3589 (1976) or French Patent Application FR-A 2701266. The types disclosed in German Patent Applications DE-A1 44 42 987 and DE-A1 195 37 001 (Henkel) and which have an average molecular weight of 800,000 to 1,200,000 d, a Brookfield viscosity (1 wt% in glycolic acid) below 5000 mPa·sec, a degree of deacetylation in a range of 80-88% and an ash content of less than 0.3 wt% are particularly preferred. In addition to chitosans as typical cationic biopolymer, anionic or nonionic derivatized chitosans, like carboxylation, succinylation or alkoxylation products are considered according to the invention, as described, e.g., in German Patent DE-C2 37 13 099 (L'Oréal) and German Patent Application DE-A1 196 04 180 (Henkel).

Phytostenol sulfates

Phytostenol sulfates, which are also considered as potentiation agents (b3) for phytostenol esters, represent known substances that can be produced, e.g., by sulfation of phytostenols with a complex of sulfur trioxide and pyridine in benzene (see J. Am. Chem. Soc. 63, 1259 (1941)). Typical examples are the sulfates of ergostenols, campestenols, stigmastenols and sitostenols, The phytostenol sulfates can be present as alkali or alkaline-earth metal salts, as ammonium, alkylammonium, alkanolammonium and/or glucammonium salts. Their sodium salts are generally used.

(Deoxy)ribonucleic acids

(Deoxy)ribonucleic acids (DNA or RNA), which are considered as the last group of potentiation agent (b4) for the phytostenol esters, are high-molecular, thread-like polynucleotides derived from 2'-deoxy-β-D-ribonucleosides or D-ribonucleosides, which in turn are constructed from equivalent amounts of a nucleic acid base and the pentoses 2-deoxy-D-ribofuranose or D-ribofuranose. DNA or RNA can contain as nucleobases the pyridine derivatives adenine and guanine, as well as the pyrimidines cytosine and thymine or uracil. In the nucleic acids the nucleobases are bonded with an N-glycoside bond to the carbon atom 1 of ribose so that adenosines, guanosines, cytidines, and thymidines are formed in individual cases. A phosphate group in the acids links the 5'-hydroxy group of the nucleoside with the 3-OH group of the

subsequent one by a phosphodiester bridge to form single-strand DNA or RNA. Because of the large ratio of length to diameter, DNA and RNA molecules have a tendency under mechanical stress, e.g., during extraction, toward rupture of the strand. For this reason the molecular weight of nucleic acids can reach 10^3-10^9 dalton. According to the invention, concentrated DNA or RNA solutions are used which are characterized by liquid crystalline behavior. Deoxy- or ribonucleic acids that are obtained from marine sources, e.g., by extraction from fish sperm and have a molecular weight in the range from 40,000 to 1,000,000 dalton are preferably used.

The active ingredient mixtures of the invention can contain the phytostenol esters (a) and the potentiation agents (b) in a weight ratio of 99:1-1:99, preferably 90:10-10:90, especially 70[sic; 75]:25 to 25:75 and most especially 60:40-40:60, in which it is merely ensured that an amount of component (a) sufficient to reduce the cholesterol content of the blood is absorbed with the application according to the invention. In a special variant of the invention, the phytostenol esters (alone or together with the potentiation agents) are encapsulated in known fashion in gelatin, in which the components (a) and optionally (b) are used in amounts of 0.1-50, preferably 1-30, especially 5-25 and most especially 10-15 wt%, referred to the weight of the gelatin capsules. Another aspect of the invention concerns the finding that encapsulation of phytostenol esters in gelatin represents an advantageous variant for oral intake of the active ingredient.

Another form of administration of phytostenol esters are suppositories, which can be introduced rectally or vaginally, and can contain gelatins, optionally combined with glycerol, or synthetic fats or waxes, polyethylene glycols and natural components, like cocoa butter, as the suppository base. It is also possible to dissolve or disperse the phytostenol esters in ordinary foods, e.g.: salad oils, dressings, mayonnaises, margarines, butter, frying fats, cocoa products, sausage and the like.

Examples 1 to 5, Comparative Examples V1 to V3

Gelatin capsules (weight about 1.5 g) with a content of 5 wt% different β-sitostenol esters and optionally vitamin E as well as 0.5 wt% radioactively labeled cholesterol were produced. To investigate the hypocholesterolemic effect, male rats were made to fast overnight (individual weight about 200 g). On the next day, the experimental animals each received a ground gelatin capsule with some salt-containing water through a stomach tube. After 3, 6, 12, 24 and 48 hours blood was taken from the animals and the content of radioactive cholesterol determined. The results, which represent the average of 10 experimental animals are summarized in Table 1. The data on reduction of radioactivity are understood with reference to a blind group of experimental animals to which only gelatin capsules with a content of 20 wt% Vitamin E and a corresponding

amount of radioactively labeled cholesterol were administered. The mixtures 1 to 5 are according to the invention, mixtures V1-V3 serve for comparison.

Table 1. Hypocholesterolemic effect (amount as wt% referred to gelatin capsule).

① Zusammensetzung/Aktivität	1	2	3	4	5	V1	V2	V3
② Konjuenfettsäure-β-sitostenolester*	5	-	-	-	-	-	-	-
③ konj. C_{12}-C_{24}-Fischfettsäure-β-sitostenolester	-	5	-	-	-	-	-	-
④ Konjuenfettsäure-β-sitostanolester*	-	-	5	-	-	-	-	-
⑤ konj. C_{12}-C_{24}-Fischfettsäure-β-sitostenolester	-	-	-	5	5	-	-	-
⑥ Laurinsäure-β-sitostanolester	-	-	-	-	-	-	-	-
⑦ Ölsäure-β-sitostanolester	-	-	-	-	-	5	-	-
⑧ Linolsäure-β-sitostanolester	-	-	-	-	-	-	5	-
Vitamin E	-	-	-	-	5	-	-	5
⑨ *Radioaktivität [%-rel]*								
- nach 3 h	95	95	95	95	95	95	95	95
- nach 6 h	80	79	78	78	75	84	82	83
⑩ *- nach 12 h*	72	70	68	67	61	76	74	73
- nach 24 h	45	45	43	43	39	51	48	47
- nach 48 h	21	20	18	17	15	30	26	25

*Fatty acid basis: Selin® CLA (Grunau/Illertissen)

Key:
1 Composition/Activity
2 Conjugated fatty acid β-sitostenol ester*
3 Conjugated C_{12}-C_{24} fish fatty acid β-sitostenol ester
4 Conjugated fatty acid β-sitostanol ester*
5 Conjugated C_{12}-C_{24} fish fatty acid β-sitostenol ester
6 Lauric acid β-sitostenol ester
7 Oleic acid β-sitostenol ester
8 Linoleic acid β-sitostenol ester
9 Radioactivity (% relative)
10 After __ hours

Claims

1. Use of esters of phytostenols with fatty acids with 6-24 carbon atoms and at least two conjugated double bonds to produce hypocholesterolemic agents.

2. Use according to Claim 1, characterized by the fact that esters of β-sitostenol or β-sitostanol are used.

3. Use according to Claims 1 and 2, characterized by the fact that esters of β-sitostenol or β-sitostanol with conjugated linolenic acid are used.

4. Use according to Claims 1 and 2, characterized by the fact that esters of β-sitostenol or β-sitostanol with conjugated fish fatty acids are used.

5. Use according to Claims 1-4, characterized by the fact that the phytostenol esters are used together with potentiation agents chosen from the group formed of tocopherols, chitosans, phytostenol esters and (deoxy)ribonucleic acids and their mixtures.

6. Use according to Claims 1-5, characterized by the fact that Vitamin E is used as potentiation agent.

7. Use according to Claims 1-6, characterized by the fact that chitosans with an average molecular weight in the range from 50,000-250,000 or 500,000-2,000,000 d are used as potentiation agents.

8. Use according to Claims 1-7, characterized by the fact that marine deoxyribonucleic acids having a molecular weight in the range from 40,000-1,000,000 d are used as potentiation agents.

9. Use according to Claims 1-8, characterized by the fact that the components (a) and optionally (b) are encapsulated in gelatin.

10. Use according to Claim 9, characterized by the fact that the phytostenol esters are used in amounts of 0.1-50 wt%, referred to the weight of the gelatin capsule.